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(a) Protein, DNA and use thereof.

(1) Disclosed are (1) a Xenopus laevis bone morphogenetic protein (BMP), (2) a DNA comprising a DNA segment coding for a Xenopus laevis BMP, (3) a transformant bearing a DNA comprising a DNA segment coding for a Xenopus laevis BMP and (4) a method for preparing the Xenopus laevis BMP which comprises culturing the described in (3), producing and accumulating the protein in a culture, and collecting the protein thus obtained. Cells transfected or transformed with the DNA allow large amounts of the Xenopus laevis BMP mature peptides to be produced which causes the advantageous production of the peptides, which promote the synthesis of proteoglycan and can also be utilized for analysis of the mechanism of organism, particularly human bone-cartilage morphogenetic reaction, and as therapeutic agents for osteoporosis.

PROTEIN, DNA AND USE THEREOF

BACKGROUND OF THE INVENTION

The present invention relates to a DNA containing a DNA segment coding for a *Xenopus laevis* bone morphogenetic protein analogous to a bone morphogenetic protein (hereinafter referred to as BMP), a precursor protein (or a precursor polypeptide) and a mature protein (or a mature polypeptide) of the *Xenopus laevis* BMP, and a method for preparing the precursor protein and the mature protein.

In this specification, the term "precursor protein" includes a protein which includes an amino acid sequence of a mature peptide *Xenopus laevis* BMP and has all or a portion of an amino acid sequence coded with a *Xenopus laevis* BMP DNA segment at the N-terminus, the C-terminus or both termini thereof.

Recently, it has been revealed that transforming growth factor-beta (TGF-beta, TGF- β) having a bone morphogenetic activity not only controls cell proliferation, but also has various biological activities such as control of cell differentiation. In particular, the bone morphogenesis-promoting activity of TGF- β has been noted, and attempts have been made to use TGF- β for treatment of fractures and osteoporosis, making use of the cartilage-bone induction activity thereof [M. Noda et al., J. Endocrinology 124, 299-309 (1990); M. E. Joyce et al., J. Bone Mineral Res. 4, S-259 (1989); and S. M. Savedijn et al., J. Biol. Chem. 261, 5693-5695 (1986)]. More recently, however, four kinds of bone morphogenetic proteins (BMPs) which are different from one another in molecular structure have been identified as a factor promoting morphogenesis of bones and cartilages. Of these four kinds, human BMP-1, human BMP-2A, human BMP-2B and human BMP-3 are novel peptides, though they are very similar in structure to TGF- β , and there has been a report that they induce morphogenesis of bones and cartilages when subcutaneously or intramuscularly implanted in animals [J. M. Wozney et al., Science 242, 1528-1534 (1989)].

The above peptides having bone morphogenetic activity are isolated and purified from bones in which the peptides are considered to be localized, or from human osteosarcoma cells (U2-Os) which are thought to produce the peptides. However, such a method has problems because the procedure is complicated and the desired peptides are obtained only in small amounts.

SUMMARY OF THE INVENTION

Important contributions will be made to future studies and medical treatment, if a similar peptide having the bone morphogenetic activity can be collected from *Xenopus laevis* and further prepared by recombinant technique. As a result, the following information was obtained thus arriving at the present invention. Namely, the present inventors first succeeded in cloning five kinds of DNA coding for BMP-2A and related DNAs (*Xenopus laevis* BMPs) and subsequently three kinds of complementary DNAs, eight kinds of DNAs in total, by using a complementary DNA of a rat inhibin α chain equally belonging to the TGF- β family as a probe. Further, the present inventors identified portions of the bases of the DNAs, clarified the amino acid sequences (see formulae (I), (II), (III), (IV) and (V) of FIG. 3 and formulae (VI), (VII) and (VIII) of FIG. 4) of the *Xenopus laevis* BMPs (referred to as B_B, M_A, C_A, A_A, A_B, X_B22, X_B23 and X_B41), and succeeded in pioneering their mass production by recombinant technique.

In accordance with the present invention, there are provided (1) a *Xenopus laevis* BMP, (2) a DNA comprising a DNA segment coding for the *Xenopus laevis* BMP, (3) a transformant bearing the DNA containing the DNA segment coding for the *Xenopus laevis* BMP and (4) a method for preparing the *Xenopus laevis* BMP which comprises culturing the transformant described in (3), producing and accumulating a protein in a culture and collecting the protein thus obtained.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows simplified restriction enzyme maps of DNA sequences containing *Xenopus laevis* BMP precursors or mature peptide DNA segments; Figs. 2(1) to 2(8) show nucleotide sequences of the DNA segments of *Xenopus laevis* BMPs, B_B, M_A, C_A, A_A, A_B, BMP-2A, BMP-2B and Vg-1, respectively, and the amino acid sequences deduced therefrom; FIG. 3 shows amino acid sequences of the *Xenopus laevis* BMPs deduced from the nucleotide sequences of the DNA segments shown in Figs. 2(1) to 2(5), comparing them with the amino acid sequences of known proteins having a bone morphogenetic activity; and

FIG. 4 shows amino acid sequences of the *Xenopus laevis* BMPs deduced from the nucleotide sequences of the cDNA segments shown in Figs. 2(6) to 2(8).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The mature *Xenopus laevis* BMP of C_A, one of the *Xenopus laevis* BMPs, of the present invention, which has a relationship to TGF- β and is a peptide consisting of 98 or 114 amino acid residues, has an amino acid sequence represented by Nos. 6 to 119 or Nos. 22 to 119 of formula (III) shown in FIG. 3. The molecular weight thereof is calculated at about 25,000, excepting sugar chains, when a dimer is formed.

The amino acid sequence of this peptide is different from that reported by Wozney et al. in 3 or 4 amino acid residues per molecule.

FIG. 3 shows amino acid sequences of five kinds of novel *Xenopus laevis* BMPs obtained in the present invention, comparing them with the amino acid sequences of known proteins having a bone morphogenetic activity. In these amino acid sequences, the same amino acid residue as with β is represented by " ", and an amino acid residue different from that of β is represented by one letter symbol based on β . CONSENSUS shown in FIG. 3 indicates amino acid residues common to all the BMPs shown in FIG. 3. The illustration of CONSENSUS results in introduction of gaps " " in the formulae in FIG. 3. Accordingly, the number representing the precursor and mature protein positions is counted excluding these lacking portions. FIG. 4 shows amino acid sequences of three kinds of novel *Xenopus laevis* BMPs deduced from cDNAs, subsequently discovered by the present inventors.

For DNA sequences, the DNA segments coding for the *Xenopus laevis* BMPs of the present invention correspond to the nucleotide sequences of formulae (I) to (B) (corresponding to B_B, M_A, C_A, A_A, X_B22 and X_B41, respectively) shown in FIG. 2 or are portions thereof. Any functional portion can be used so long as bone morphogenetic activity is not lost. Wozney et al. reports the amino acid sequences, but does not elucidate the nucleotide sequences. As used herein the term "Correspond" permits conservative additions, deletions and substitutions. Preferably, the DNA segments coding for the BMPs of the present invention have the nucleotide sequences of formulae (1) to (B).

With respect to the portion relating to the mature BMPs (the amino acid sequence represented by Nos. 15 to 130 of formula (I) shown in FIG. 3, the amino acid sequence represented by Nos. 14 to 127 of formula (II) shown in FIG. 3, the amino acid sequence represented by Nos. 6 to 119 or Nos. 22 to 119 of formula (III) shown in FIG. 3, the amino acid sequence represented by Nos. 6 to 65 of formula (IV) shown in FIG. 3, the amino acid sequence represented by Nos. 282 to 398 or Nos. 298 to 388 of formula (V) shown in FIG. 3, the amino acid sequence represented by Nos. 288 to 401 or Nos. 304 to 401 of formula (VI) shown in FIG. 4, or the amino acid sequence represented by Nos. 328 to 426 of formula (VII) shown in FIG. 4], the DNA sequences of the present invention differ from the DNA sequence of TGF- β , and therefore are novel.

As the DNA sequences coding for the BMP mature peptides of the present invention, any DNA sequences may be used as long as they contain nucleotide sequences coding for the amino acid sequences of the BMP mature peptides. For example, DNA sequences corresponding to the nucleotide sequences represented by formulae (1) to (8) or portions thereof are preferably used. More preferably the DNA sequences contain the nucleotide sequences represented by formulae (1) to (8).

The nucleotide sequences represented by formulae (1) to (8) are the *Xenopus laevis* BMP DNA sequences obtained in the present invention. Examples of the nucleotides coding for the *Xenopus laevis* BMP amino acid sequences represented by formulae (1) to (VII) include Nos. 693 to 1050 of formula (1), Nos. 134 to 475 of formula (2), Nos. 435 to 728 of formula (3), Nos. 183 to 356 of formula (4), Nos. 149 to 328 of formula (5), Nos. 249 to 1442 of formula (6), Nos. 104 to 1306 of formula (7) and Nos. 88 to 1363 of formula (8).

An expression vector having the DNA sequence containing the nucleotide sequence coding for the BMP of the present invention can be prepared, for example, by the following process:

- (a) Messenger RNA (mRNA) is isolated from BMP-producing cells.
- (b) Single stranded complementary DNA (cDNA) is synthesized from the mRNA, followed by synthesis of double stranded DNA.
- (c) The complementary DNA is introduced in a cloning vector such as a phage or a plasmid.
- (d) Host cells are transformed with the recombinant phage or plasmid thus obtained.
- (e) After cultivation of the transformant thus obtained, the plasmid or the phage containing the desired DNA is isolated from the transformant by an appropriate method such as hybridization with a DNA probe coding for a portion of the BMP or immunoassay using an anti-BMP antibody.

- (i) The desired cloned DNA sequence is cut out from the recombinant DNA.
 (g) The cloned DNA sequence or a portion thereof is ligated downstream from a promoter in the expression vector.
- The mRNAs coding for the BMPs can be obtained from various BMP-producing cells such as ROS cells. Methods for preparing the mRNAs from the BMP-producing cells include the guanidine thiocyanate method [U. M. Chirgwin et al., *Biochemistry* 18, 5294 (1979)]. Using the mRNA thus obtained as a template, cDNA is synthesized by use of reverse transcriptase, for example, in accordance with the method of H. Okuyama et al., *Molecular and Cellular Biology* 2, 161 (1982); *ibid.* 3, 280 (1983). The cDNA thus obtained is introduced into the plasmid.
- The plasmids into which the cDNA is introduced include, for example, pBR322 [*Gene* 2, 95 (1977)], pBR325 [*Gene* 4, 121 (1978)], pUC12 [*Gene* 19, 259 (1982)] and pUC13 [*Gene* 19, 259 (1982)]; each derived from *Escherichia coli*, and pUB110 derived from *Bacillus subtilis* [*Biochemical and Biophysical Research Communication* 112, 678 (1983)]. However, any other plasmids can be used as long as they are replicable and growable in the host cells. Examples of the phage vectors into which the cDNA may be introduced include Agull [R. Young and R. Davis, *Proc. Natl. Acad. Sci. U.S.A.* 80, 1194 (1983)]. However, any other phage vectors can be used as long as they are growable in the host cells.
- Methods for introducing the cDNA in the plasmid include, for example, the method described in T. Maniatis et al., *Molecular Cloning*, Cold Spring Harbor Laboratory, p.239 (1982). Methods for introducing the cDNA in the phage vector include, for example, the method of T. V. Hyun et al., *DNA Cloning. A Practical Approach* 1, 49 (1985).
- The plasmid thus obtained is introduced into the appropriate host cell such as *Escherichia* and *Bacillus*. Examples of *Escherichia* described above include *Escherichia coli* K12DH1 [*Proc. Natl. Acad. Sci. U.S.A.* 60, 160 (1963)], M103 [*Nucleic Acids Research* 9, 305 (1981)], J4221 [*Journal of Molecular Biology* 120, 517 (1978)], HB101 [*Journal of Molecular Biology* 41, 459 (1969)] and C500T [*Genetics* 39, 240 (1955)].
- Examples of *Bacillus* described above include *Bacillus subtilis* M114 [*Gene* 24, 255 (1983)] and 207.
- 21 [*Journal of Biochemistry* 95, 87 (1984)].
- Methods for transforming the host cell with the plasmid include, for example, the calcium chloride method or the calcium chloride method described in T. Maniatis et al., *Molecular Cloning*, Cold Spring Harbor Laboratory, p.249 (1982).
- When the phage vector is used, for example, the phage vector can be transduced into multiplied *Escherichia coli* using the *in vitro* packaging method.
- Xenopus laevis* cDNA libraries containing *Xenopus laevis* BMP cDNA can be obtained by numerous techniques well known in the art, including purchasing them from the market, though obtainable by the methods described above. For example, the cDNA library of *Xenopus laevis* is available from Clontech Laboratories, Inc., U.S.A.
- Methods for cloning the *Xenopus laevis* BMP DNA from the *Xenopus laevis* DNA library include, for example, the plaque hybridization method using phage vector λcharon 28A and rat inhibitor (activin) βA cDNA as probes [T. Maniatis et al., *Molecular Cloning*, Cold Spring Harbor Laboratory, (1982)].
- The *Xenopus laevis* BMP DNA thus cloned is subcloned in plasmids such as pBR322, pUC12, pUC13, pUC18, pUC118 and pUC119 to obtain the *Xenopus laevis* BMP DNA, if necessary.
- The nucleotide sequence of the DNA sequence thus obtained is determined, for example, by the Maxam-Gilbert method [A. M. Maxam and W. Gilbert, *Proc. Natl. Acad. Sci. U.S.A.* 74, 580 (1977)] or the dideoxy method [J. Messing et al., *Nucleic Acids Research* 6, 303 (1978)], and the existence of the *Xenopus laevis* BMP DNA is confirmed in comparison with the known amino acid sequence.
- As described above, the DNA sequences [*Xenopus laevis*] BMP DNAs represented by formulae (1) to (8) coding for the *Xenopus laevis* BMPs are obtained.
- Fig. 1 shows the restriction enzyme fragment maps of the DNA sequences containing the DNA segments coding for the *Xenopus laevis* BMPs obtained in Example 1 described below. Fig. 2 shows the nucleotide sequences represented by formulae (1) to (8) of the DNA sequences as determined by the dideoxy method, and Figs. 3 and 4 show the amino acid sequences represented by formulae (1) to (8) and formulae (VII) to (VIII), respectively, which were ascertained from the above nucleotide sequences.
- The DNA sequence coding for the *Xenopus laevis* BMP cloned as described above can be used as it is, or after digestion with a restriction enzyme if desired, depending on the intended use.
- A region intended to be expressed is cut out from the cloned DNA and ligated downstream from the promoter in a vehicle (vector) suitable for expression, whereby the expression vector can be obtained.

The DNA sequence has ATG as a translation initiating codon at the 5'-terminus thereof and may have TAA, TGA or TAG as a translation terminating codon at the 3'-terminus. The translation initiating codon and translation terminating codon may be added by use of an appropriate synthetic DNA adaptor. The promoter is further ligated in the upstream thereof for the purpose of expressing the DNA sequence.

Examples of the vectors include the above plasmids derived from *E. coli* such as pBR322, pBR325, pUC12 and pUC13, the plasmids derived from *B. subtilis* such as pUB110, pTP5 and pC184, plasmids derived from yeast such as pSH19 and pSH15, bacteriophage such as λphage, and animal viruses such as retroviruses and vaccinia viruses.

As the promoters used in the present invention, any promoters are appropriate as long as they are suitable for expression in the host cells selected for the gene expression.

When the host cell used for transformation is *Escherichia*, it is preferable that a trp promoter, a lac promoter, a recA promoter, a λPL promoter, a λPP promoter, etc. are used. When the host cell is *Bacillus*, it is preferable that a PHO5 promoter, a PGK promoter, a GAP promoter, an ADH promoter, etc. are used. In particular, it is preferable that the host cell is *Escherichia* and the promoter is the trp promoter or the λPL promoter.

When the host cell is an animal cell, an SV-40 derived promoter, a retrovirus promoter, a metallothionein promoter, a heat shock promoter, etc. are each usable.

An enhancer, a certain DNA sequence important for promoter activity in a cell, is also effectively used for expression.

By using the vector containing the DNA sequence coding for the *Xenopus laevis* BMP mature peptide thus constructed, the transformant is prepared.

The host cell includes, for example, *Escherichia*, *Bacillus*, yeast and animal cells.

Specific examples of the above *Escherichia* and *Bacillus* include strains similar to those described above.

Examples of the above yeast include *Saccharomyces cerevisiae* AH22, AH22R-, NA87-1A and DKD-5D.

Experiments of animal cells include monkey cell COS-7, Vero, Chinese hamster cell (CHO), mouse L cell and human FL cell.

The transformation of the above *Escherichia* is carried out, for example, according to the method described in *Proc. Natl. Acad. Sci. U.S.A.* 69, 2170 (1972) or *Gene* 17, 107 (1982).

The transformation of the above *Bacillus* is conducted, for example, according to the method described in *Molecular & General Genetics* 168, 111 (1979).

The transformation of the yeast is carried out, for example, according to the method described in *Proc. Natl. Acad. Sci. U.S.A.* 75, 1929 (1978).

The transformation of the animal cells is carried out, for example, according to the method described in *Virology* 52, 456 (1973).

Thus, there is obtained the transformant transformed with the expression vector containing the DNA sequence coding for the *Xenopus laevis* BMP mature peptide.

When bacterial transformants are cultured, a liquid medium is particularly suitable as a medium used for culture. Carbon sources, nitrogen sources, inorganic compounds and others necessary for growth of the transformant are contained therein. Examples of the carbon sources include glucose, dextrose, soluble starch and sucrose. Examples of the nitrogen sources include inorganic or organic materials such as ammonium salts, nitrates, corn steep liquor, peptone, casein, meat extracts, soybean meal and potato extract solution. The inorganic compounds include, for example, calcium chloride, sodium dihydrogen phosphate and magnesium chloride. Yeast extract, vitamins, growth promoting factors and so on may be further added thereto.

The pH of the medium is preferably about 5 to 8.

As the medium used for cultivation of *Escherichia*, there is preferred, for example, M9 medium containing glucose and Casamino Acids (Miller, *Journal of Experiments in Molecular Genetics* 431-433, Cold Spring Harbor Laboratory, New York, 1972). In order to make the promoter act efficiently, a drug such as 3β-indolylacrylic acid may be added thereto if necessary.

When the host cell is *Escherichia*, the cultivation is usually carried out at about 15 to 43°C for about 3 to 24 hours, with aeration or agitation if necessary.

When the host cell is *Bacillus*, the cultivation is usually carried out at about 30 to 40°C for about 6 to 24 hours, with aeration or agitation if necessary.

When yeast transformants are cultured, there is used, for example, Burkholder minimum medium [K. L. Bostian et al., *Proc. Natl. Acad. Sci. U.S.A.* 77, 4505 (1980)] as the medium. The pH of the medium is preferably adjusted to about 5 to 8. The cultivation is usually carried out at about 20 to 35°C for about 24,

to 72 hours, with aeration or agitation if necessary.

When animal cell transfromants are cultured, examples of the media include MEM medium containing about 5 to 20% fetal calf serum [Science 122, 501 (1952)], DMEM medium [Virology 8, 398 (1959)], RPMI 1640 medium [Journal of the American Medical Association 189, 519 (1967)] and 199 medium [Proceeding of the Society for the Biological Medicine 73, 1 (1950)]. The pH is preferably about 6 to 8. The cultivation is usually carried out at about 30 to 40°C for about 15 to 60 hours, with aeration or agitation if necessary.

The above *Xenopus laevis* BMP mature peptide can be isolated and purified from the culture described above, for example, by the following method.

10 When the *Xenopus laevis* BMP mature peptide is to be extracted from the cultured cells, the cells are collected by methods known in the art after cultivation. Then, the collected cells are suspended in an appropriate buffer solution and disrupted by ultrasonic treatment, lysozyme and/or freeze-thawing. Thereafter, a crude extracted solution of the *Xenopus laevis* BMP mature peptide is obtained by centrifugation or filtration. The buffer solution may contain a protein denaturant such as urea or guanidine hydrochloride, or a surface-active agent such as Triton X-100.

15 When the *Xenopus laevis* BMP precursor protein or mature peptide is secreted in the culture solution, a supernatant is separated from the cells by methods known in the art after the conclusion of cultivation, and then collected.

20 The separation and purification of the *Xenopus laevis* BMP precursor protein or mature peptide contained in the culture supernatant or the extracted solution thus obtained can be performed by an appropriate combination of known separating and purifying methods. This known separating and purifying methods include methods utilizing solubility such as salt precipitation and solvent precipitation, methods mainly utilizing a difference in molecular weight such as dialysis, ultrafiltration, gel filtration and SDS-polyacrylamide gel electrophoresis, methods utilizing a difference in electric charge such as ion-exchange column chromatography, methods utilizing specific affinity such as affinity chromatography, methods utilizing a difference in hydrophobicity such as reverse phase high performance liquid chromatography and methods utilizing a difference in isoelectric point such as isoelectric-focusing electrophoresis. Methods using an antibody to a fused protein expressed by fusing BMP complementary DNA or DNA with *E. coli*-derived DNA lacZ can also be used.

25 Illustrative examples of the methods for expressing the BMP in the present invention include methods in which genes are introduced into CHO cells to produce the BMP in large amounts as described in Wang et al. Proc. Natl. Acad. Sci. U.S.A. 80, 2220-2224 (1983).

30 The activity of the *Xenopus laevis* BMP precursor protein or mature peptide thus formed can be measured by an enzyme immunoassay using a specific antibody. If the products have a bone mor. photogenic activity, this activity may also be measured as an index.

35 The cells, such as animal cells or *E. coli*, transfected or transformed with the DNA sequences of the present invention allow large amounts of the *Xenopus laevis* BMP mature peptides to be produced. Hence, the production of these peptides can be advantageously achieved.

It has become clear that the *Xenopus laevis* BMP mature peptides prepared here promote the synthesis of proteoglycan which is a main component of a cartilage matrix, and the peptides can also be utilized for

40 analysis of the mechanism of organism, particularly human bone-cartilage morphogenetic reaction, and as therapeutic agents for fracture or osteoporosis.

In such instances one would administer an effective amount of the protein to a mammal. An effective amount is the amount of protein needed to promote the synthesis of proteoglycan in cartilage cells.

45 Typically, this ranges from 0.001 to 35 ug per kg body weight. The precise amount for a particular purpose can readily be determined empirically by the person of ordinary skill in the art based upon the present disclosure.

When one uses the protein for therapeutic purpose care is taken to purify it and render it substantially free of bacteria and pyrogens. This can be done by standard methods.

50 When the BMPs are used as therapeutic agents for fracture or osteoporosis, they can be administered parenterally in the forms of solutions, injections and ointments, solely or in combination with pharmaceutically acceptable additional components, such as vehicles, binders, dispersants, plasticizers or diluents.

The preferable administration forms include (1) administration of the agent to cuts surface near a diseased part, (2) injection of the agent into a diseased part, (3) discision of a diseased part followed by direct administration of the agent thereto. The preferable dose for adult people is 0.1 to 2000 ug more, preferably 20 to 400 ug for adult people once a day. The preferable dose in osteoporosis for adult people is 0.1 to 200 ug once a day, for about one to 30 days. The concentration of the therapeutic agent is, preferably, 0.001 to 0.2% in the form of a solution, 0.001 to 0.2% in the form of an injections, and

0.001 to 0.2% in the form of an ointment.

There have been described above in detail the cloning of the DNA sequences coding for the *Xenopus laevis* BMPs, the preparation of the expression vectors for the *Xenopus laevis* BMP mature peptides, the production of the transfromants by using the transfromants and their utility.

When nucleotides, amino acids and so on are indicated by the abbreviations in this specification and drawings, the abbreviations adopted by IUPAC-IUB Commission on Biochemical Nomenclature or commonly used in the art are employed. For example, the following abbreviations are used. When the amino acids are capable of existing as optical isomer, the L-forms are represented unless otherwise specified.

A : Adenine
T : Thymine
G : Guanine
C : Cytosine
RNA : Ribonucleic acid

mRNA : Messenger ribonucleic acid
dATP : Deoxyadenosine triphosphate
dGTP : Deoxyguanosine triphosphate

dCTP : Deoxycytidine triphosphate
dTTP : Deoxythymidine triphosphate

ATP : Adenosine triphosphate
EDTA : Ethylenediaminetetraacetic acid

SDS : Sodium dodecyl sulfate
Glu or E : Glutamic acid
Asp or D : Aspartic acid

55 Lys or K : Lysine
Val or V : Valine
Leu or L : Leucine
Ile or I : Isolucine
Ser or S : Serine
Thr or T : Threonine
Cys or C : Cysteine

Met or M : Methionine
His or H : Histidine

Glu or E : Glutamic acid
Asp or D : Aspartic acid

Tyr or Y : Tyrosine
Trp or W : Tryptophan

Pro or P : Proline
Asn or N : Asparagine
Gln or Q : Glutamine

With respect to the *Xenopus laevis* BMP mature peptides of the present invention, a portion of the amino acid sequence may be modified, namely there may be addition, elimination or substitution with other amino acids as long as the bone morphogenetic activity is not lost.

The present invention will hereinafter be described in detail with the following Examples. It is understood of course that these Examples are not intended to limit the scope of the invention.

45 Transfromants *E. coli* HB101/pXa3 (coding for protein M3), *E. coli* HB101-pXa4 (coding for protein A4).

50 *E. coli* HB101/pXa5 (coding for protein A5), *E. coli* HB101-pXa9 (coding for protein B9) and *E. coli* HB101-pXa14 (coding for protein C4) each obtained in Example 1 described below were deposited with the Institute for Fermentation, Osaka, Japan (IFO) under the accession numbers IFO 14928, IFO 14929, IFO 14930, IFO 14931 and IFO 14932, respectively, on August 28, 1989. These transformants were also deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan (FRI) under the Budapest Treaty under the accession numbers FERM BP-2578, FERM BP-2579, FERM BP-2580, FERM BP-2581 and FERM BP-2582, respectively, on September 2, 1989.

The transfromants *E. coli* HB101-pXa22 (coding for *Xenopus laevis* BMP-2A), *E. coli* HB101-pXa23

(coding for Xenopus laevis BMP-2B) and E. coli HB101/pXbr41 (coding for protein Xenopus laevis Vgr-1) each obtained in Example 2 described below were deposited with the Institute for Fermentation, Osaka, Japan (IFO), under the accession numbers IFO 15080, IFO 15081 and IFO 15082, respectively, on August 10, 1990. These transformants were also deposited with the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan (FRI) under the Budapest Treaty under the accession numbers FERM BP-3066, FERM BP-3065 and FERM BP-3067, respectively, on August 16, 1990.

10 Example 1

Preparation of Xenopus laevis Liver-Derived DNA Library

15 (1) Preparation of Xenopus laevis Chromosome DNA

The liver (1 g) of Xenopus laevis was powdered in liquid nitrogen, and 10 ml of buffer (1) [100 µg/ml proteinase K, 0.5% SDS, 0.5 mM EDTA (pH 8.0)] was added thereto, followed by incubation at 50°C for 2 hours. The resulting DNA sample was treated with phenol, and then dialyzed against buffer (2) [10 mM EDTA, 10 mM NaCl, 50 mM Tris-HCl (pH 8.0)] to remove phenol. RNase was added thereto to a final concentration of 100 µg/ml, and the mixture was incubated at 37°C for 3 hours, followed by phenol treatment twice. The aqueous layer was dialyzed against buffer (3) [1 mM EDTA, 10 mM Tris-HCl (pH 8.0)]. Thus, about 1 mg of liver-derived chromosome DNA was obtained. This DNA (10 µg) was partially cleaved with restriction enzyme Sau3AI, and the resulting product was subjected to equilibrium density gradient centrifugation using CsCl. Fractions containing DNA fragments having lengths of 10 to 20 kb were selected and introduced into fragments obtained by cleaving phage charon 28 DNA with BamHI and used as a vector. This reaction called "ligation" was conducted at 15°C for 18 hours. The charon 28 vector into which the Xenopus laevis chromosome DNA was thus introduced was contained in a phage head (in vitro packaging). This procedure was carried out by using a commercial packaging kit (Gigapack Gold, Stratagene). This recombinant phage was amplified by infection with E. coli LE392. Specifically, the phage was mixed with excess LE392 to allow LE392 to adsorb the phage at 37°C for 10 minutes. Then, the mixture was plated on NZYM medium (containing 13% agar), followed by incubation overnight.

20 (2) Screening

The total number of the phage clones was estimated to be about 1,000,000 from the number of the plaques produced in a dish. As a probe (DNA used for detection of a desired gene by hybridization), there was used rat activin $\beta\alpha$ cDNA [Molecular Endocrinology 1, 388-396 (1987)] labeled with ^{32}P by a random priming method. The plaques transcribed from the dish to a nitrocellulose membrane were returned to neutrality (0.2 M Tris, 0.6 M NaCl, pH 7.4) through alkali treatment (immersion in 0.1 N NaOH, 0.8 M NaCl for 30 seconds). After completion of the treatment described above, the membrane was heated in a vacuum (for 30 seconds). After heating, the membrane was immersed in a hybridization solution (50% formamide, 5 X Denhardt's solution, 5 X SSPE, 0.1% SDS, 100 µg/ml denatured salmon sperm DNA) to incubate it at 42°C for 4 hours. Then, the membrane was allowed to stand in the mixture solution of the above hybridization solution and the DNA probe at 60°C overnight. This procedure was carried out in a plastic bag. The next day, the nitrocellulose membrane was taken out of the bag, and washed with a solution of 2 X SSC and 0.1% SDS for 15 minutes and with a solution of 0.1 X SSC and 0.1% SDS for 15 minutes. Increasing the temperature stepwise, until the cpm value of the membrane reached about 1,000 cpm. After washing, the washing solution was removed by filter paper, and then the membrane was subjected to autoradiography. The plaque containing the desired gene was identified by exposure of a Fuji X-ray film. The genes were cloned by repetition of the above plaque hybridization.

25 20 X SSC contains 0.3 M sodium citrate (pH 7.0) and 3 M NaCl; 20 X SSPE contains 0.2 M sodium phosphate, 20 mM EDTA and 3 M NaCl; and Denhardt's solution contains 1% Ficoll, 1% polyvinylpyrrolidone and 1% BSA (Pentex Fraction V).

(3) Determination of Nucleotide Sequence (Sequencing)

All of the five isolated clones A4, A5, B9, C4 and M3 were each subcloned into plasmid pUC19. In subcloning each clone into plasmid pUC19, subcloning was carried out utilizing a restriction enzyme recognition site which produced a fragment hybridized with the probe for each clone. However, for cloning clone A4, a commercial BglII linker was used to ligate a SmaI site.

The plasmids were each transformed into competent cell HB101 (E. coli) prepared by the rubidium chloride method to obtain five kinds of transformants E. coli HB101/pXar3 (coding for protein A5), E. coli HB101/pxar4 (coding for protein A4), E. coli HB101/pxar5 (coding for protein B9), E. coli HB101/pXar6 (coding for protein C4), respectively.

For determination of the nucleotide sequence, a deletion mutant of each clone was prepared, and the shortest of fragment hybridized with the probe was selected. The nucleotide sequence was determined from pUC19 by the direct Sanger method (or the dideoxy method). For translation of the nucleotide sequence or for screening of homology, a software for genetic analysis (GENETYX, Nippon SDC) was used.

15 (2) Preparation of Xenopus laevis Unfertilized Egg-Derived DNA Library

20 (1) Preparation of Xenopus laevis BMP-2A Probe

A probe was prepared by fragmentation of chromosome DNA Xar14 coding for Xenopus laevis BMP-2A with restriction enzymes PstI and HindIII, and three kinds of cDNAs, Xbr22, Xbr23 and Xbr4 were isolated by screening of a Xenopus laevis unfertilized egg cDNA library by a hybridization method. The comparison with the structure of the Xenopus laevis BMP-2A chromosome DNA already isolated revealed that Xbr22, Xbr23 and Xbr4 coded for proteins having homology with Xenopus laevis BMP-2A, Xenopus laevis BMP-2B and mouse Vgr-1 reported by Lyon et al. [Proc. Natl. Acad. Sci. U.S.A., 80, 4554-4558 (1983)], respectively.

The Xenopus laevis unfertilized egg cDNA library was provided by the Salk Institute (C. Kintner). This

Homology at Nucleic Acid Level						
TYX nucleotide	Rat Act. $\beta\alpha$, %	Rat Act. $\beta\alpha$, %	Human TGF $\beta2$, %	xVgr %	M3 %	A4 %
A5	70.3 (101)	47.5 (314)	43.8 (189)	48.5 (171)	54.7 (258)	63.7 (328)
A4	69.5 (105)	-	-	-	55.4 (251)	-
M3	63.8 (332)	53.9 (672)	30.1 (689)	-	-	-

In the above table, numerical values in parentheses indicate the length compared (bp).

40 (3) Preparation of Xenopus laevis Unfertilized Egg-Derived DNA Library

45 Example 2

In the above table, numerical values in parentheses indicate the length compared (bp).

40 (1) Preparation of Xenopus laevis BMP-2A Probe

A probe was prepared by fragmentation of chromosome DNA Xar14 coding for Xenopus laevis BMP-2A with restriction enzymes PstI and HindIII, and three kinds of cDNAs, Xbr22, Xbr23 and Xbr4 were isolated by screening of a Xenopus laevis unfertilized egg cDNA library by a hybridization method. The comparison with the structure of the Xenopus laevis BMP-2A chromosome DNA already isolated revealed that Xbr22, Xbr23 and Xbr4 coded for proteins having homology with Xenopus laevis BMP-2A, Xenopus laevis BMP-2B and mouse Vgr-1 reported by Lyon et al. [Proc. Natl. Acad. Sci. U.S.A., 80, 4554-4558 (1983)], respectively.

library was prepared based on λ g10. This recombinant phage was amplified by infection with *E. coli* NIM514. Specifically, the phage was mixed with excess NIM514 to allow NIM514 to adsorb the phage at 37°C for 10 minutes. Then, the mixture was plated on NZYM medium (containing 13% agar), followed by incubation overnight.

(2) Screening

The total number of the phage clones was estimated to be about 1,200,000 (from the number of the plaques produced in a dish). As a probe (DNA used for detection of a desired gene by hybridization), there was used a DNA fragment (185 bp) obtained by cleaving Xan14 with restriction enzymes PstI and Klenow and labeled with 32 P by a random priming method. The plaques transcribed from the dish to a nitrocellulose membrane were returned to neutrality (0.2 M Tris, 0.6 M NaCl, pH 7.4) through alkali treatment (immersion in 0.1 N NaOH, 0.6 M NaCl for 30 seconds). After completion of the treatment described above, the membrane was heated in a vacuum thermostat at 80°C for 1 hour. After heating, the membrane was immersed in a hybridization solution (50% formamide, 5 X Denhardt's solution, 5 X SSPE, 0.1% SDS, 100 μ g/ml denatured salmon sperm DNA) to incubate it at 42°C for 4 hours. Then, the membrane was allowed to stand in the mixture solution of the above hybridization solution and the DNA probe at 60°C overnight. This procedure was carried out in a plastic bag. The next day, the nitrocellulose membrane was taken out of the bag, and washed with a solution of 2 X SSC and 0.1% SDS for 15 minutes, increasing the temperature stepwise, until the cpm value of the membrane reached about 1,000 cpm. After washing, the washing solution was removed by filter paper, and then the membrane was subjected to autoradiography. The plaque containing the desired gene was identified by exposure of a Fuji X-ray film. The genes were cloned by repetition of the above plaque hybridization.

20 X SSC contains 0.3 M sodium citrate (pH 7.0) and 3 M NaCl; 20 X SSPE contains 0.2 M sodium phosphate, 20 mM EDTA and 3 M NaCl; and Denhardt's solution contains 1% Ficoll, 1% polyvinylpyrrolidone and 1% BSA (Pentex Fraction V).

(3) Determination of Nucleotide Sequence (Sequencing)

All of the three isolated clones Xbr22, Xbr23 and Xbr41 were each subcloned into plasmid pUC19. In subcloning each clone into plasmid pUC19, subcloning was carried out utilizing a restriction enzyme recognition site which produced a fragment hybridized with the probe for each clone.

25 The plasmids were each transformed into competent cell HB101 (*E. coli*) prepared by the rubidium chloride method to obtain three kinds of transformants *E. coli* HB101/pXbr22 (coding for *Xenopus laevis* BMP-2A), *E. coli* HB101/pXbr23 (coding for *Xenopus laevis* BMP-2B) and *E. coli* HB101/pXbr41 (coding for protein *Xenopus laevis* Vgr-1), respectively.

For determination of the nucleotide sequence, a deletion mutant of each clone was prepared, and the shortest fragment that hybridized with the probe was selected. The nucleotide sequence was determined from pUC19 by the direct Sanger method (or the dideoxy method).

For translation of the nucleotide sequence to an amino acid sequence or for screening of homology, a software for genetic analysis (GENETYX, Nippon SDC) was used. Figs. 2(6) to 2(8) show the respective nucleotide sequences, and Figs. 4(VI) to 4(VIII) show the respective amino acid sequences.

Example 3

50 In order to examine the biological activity of the *Xenopus laevis* BMP-related gene products, each of Xbr22, Xbr23 and Xbr41 cDNAs was inserted into expression vector pCDM8 (Invitrogen, U.S.A.) (or animal cells, and expressed in a COS cell (African green monkey kidney cell). The resulting culture supernatant was used for determination of the biological activity.

55 Each of the Xbr22, Xbr23 and Xbr41 cDNAs to which XbaI linkers were ligated at both ends thereof was inserted into the XbaI restriction enzyme-cleaving site of pCDM8 to use it for transfection (introduction of DNA). 3×10^5 cells were subcultured in a 100 mm diameter plastic dish, and the medium was removed after 24 hours, followed by washing once with 10 ml of TBS (Tris-buffered saline), 300 μ l of a DNA solution (1.5 μ g DNA) diluted with TBS was mixed with 300 μ l of a 0.1% DEAE-dextran solution, and the combined

solution was added dropwise to the cells. After standing at ordinary temperature for 15 minutes, the cells were washed once with 300 μ l of TBS, and then incubated in Dulbecco's modified Eagle's medium (DMEM, containing 10% FBS, 100 μ g/ml penicillin, 100 μ g/ml streptomycin and 100 μ M chloroquine). After 3 hours, the cells were washed twice with TBS and incubated in DMEM (containing 10% FBS, 100 μ g/ml penicillin and 100 μ g/ml streptomycin). After 24 hours, the cells were washed three times with TBS and incubated in DMEM (containing 100 μ g/ml penicillin and 100 μ g/ml streptomycin) for 4 days, followed by recovery of the medium. The recovered medium was centrifuged at 2,000 rpm for 5 minutes to obtain a culture supernatant.

The culture supernatant thus obtained was used for determination of the biological activity as a sample containing *Xenopus laevis* BMP2-A, BMP2-B or protein Vgr-1. Namely, each of the samples was added to the medium of rabbit chondrocytes in monolayer cultures [Y. Kato et al., *Exp. Cell Res.*, 130, 73-81 (1980); Y. Kato et al., *J. Biol. Chem.*, 265, 5903-5909 (1990)] to examine their effect on the synthesis of proteoglycan, the main component of cartilage matrix. As a result, the control in which the COS cell was transfected with the expression vector alone and the medium conditioned by untreated COS cells did not affect the synthesis of proteoglycan, as shown in the following table. In contrast, the above three kinds of proteins obtained in the present invention strongly promoted the synthesis of proteoglycan by the cartilage cells. The maximum activity of *Xenopus laevis* BMP-2A, BMP-2B and Vgr-1 was stronger than that of TGF- β . The synthesis of proteoglycan was determined by measuring 35 S-sulfate incorporation into glycosaminoglycans [Y. Kato et al., *Exp. Cell Res.*, 130, 73-81 (1980); Y. Kato et al., *J. Biol. Chem.*, 265, 5903-5909 (1990)]. These results show that the BMPs of *Xenopus laevis* promote the differentiation of cartilages, and suggest that the BMPs of other animals have similar effects. The BMPs are therefore expected to be applied to therapeutic agents for healing acceleration of fractures and for various diseases of cartilages and bones (such as arthritis and osteoporosis).

25 * Kind of Cell
Rabbit costal chondrocytes maintained on 6-mm diameter plastic wells.
* Kind of Marker

30 * Kind of Medium
S IucCu/ in 100 μ l medium per well

A 1:1 (V/V) mixture of DMEM and Ham's F-12 medium supplemented with 0.3% fetal bovine serum.

No.	Additive	Count	Mean ± S.D.	% to Control
1	Control	5183	4228	4269
2	xBMP2A 1.5ul	4565	4277	5089
3	xBMP2A 1.75ul	2362	2749	2382 ± 185
4	xBMP2A 1.105ul	12198	15502	21891
5	xBMP2B 1.5ul	10004	9738	8848
6	xBMP2B 1.75ul	3171	206	3219
7	xBMP2B 1.105ul	11315	9750	13139
8	xVgr-1 1.5ul	12426	13457	13324
9	xVgr-1 1.75ul	5188	2333	4416
10	xVgr-1 1.105ul	7486	8334	7202
11	pCDMB 5ul	15286	15845	13032
12	pCMDB 1ul	3604	294	2927
13	DNA(+)5ul	2637	4219	3428 ± 781
14	DNA(-)1ul	3625	4050	4714
15	DME 5ul	5695	4857	5176 ± 519
16	DME 1ul	3614	8663	3850
17	TGF-β1 3ng/ml	4384	3074	5478 ± 2468
18	Insr. 5 μg/ml	10058	12474	10922
19	Insr. 3 g/ml	19431	20476	22748
		25068	27935	24985
		13620	15378	11987
		11240	12698	12686
pCDMB: A culture solution of the cells into which pCDMB is introduced as a vector				
DNA(+): A culture solution which is in contact with the cells, which do not produce				
The BMPs				
DME: A solution which is not in contact with the cells				
Ins.: Insulin				

Fig. 3, an amino acid sequence represented by Nos. 282 to 398 or Nos. 304 to 401 of formula (VII) shown in Fig. 3, an amino acid sequence represented by Nos. 288 to 398 or Nos. 304 to 401 of formula (VII) shown in Fig. 4, or an amino acid sequence represented by Nos. 328 to 428 of formula (VIII) shown in Fig. 4.

3. A Xenopus laevis bone morphogenetic protein in accordance with claim 1, wherein said protein is a precursor protein containing an amino acid sequence corresponding to amino acid sequence represented by formula (I), (III), (III), (IV) or (V) shown in Fig. 3, or formula (VI), (VII) or (VIII) shown in Fig. 4.

4. A DNA comprising a DNA segment coding for a Xenopus laevis bone morphogenetic protein, corresponding to the nucleotide sequence represented by formula (1), (2), (3), (4), (5), (6), (7) or (8) shown in Fig. 2, or a portion thereof.

5. A transformant bearing a DNA comprising a DNA segment coding for a Xenopus laevis bone morphogenetic protein.

6. A transformant in accordance with claim 5, which has the characteristics of Escherichia coli HB101/pXar3 (FERM BP-2578).

7. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar4 (FERM BP-2579).

8. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar5 (FERM BP-2580).

9. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar6 (FERM BP-2581).

10. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar7 (FERM BP-2582).

11. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar8 (FERM BP-2583).

12. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar9 (FERM BP-2584).

13. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar10 (FERM BP-2585).

14. A transformant in accordance with claim 6, which has the characteristics of Escherichia coli HB101/pXar11 (FERM BP-2586).

15. A method for preparing a Xenopus laevis bone morphogenetic protein which comprises culturing a transformant bearing a DNA comprising a DNA segment coding for the protein, producing and accumulating the protein in culture, and collecting the protein thus obtained.

16. A composition for therapy of fracture or osteoporosis which contains an effective amount of a Xenopus laevis bone morphogenetic protein.

Experiments Procedure

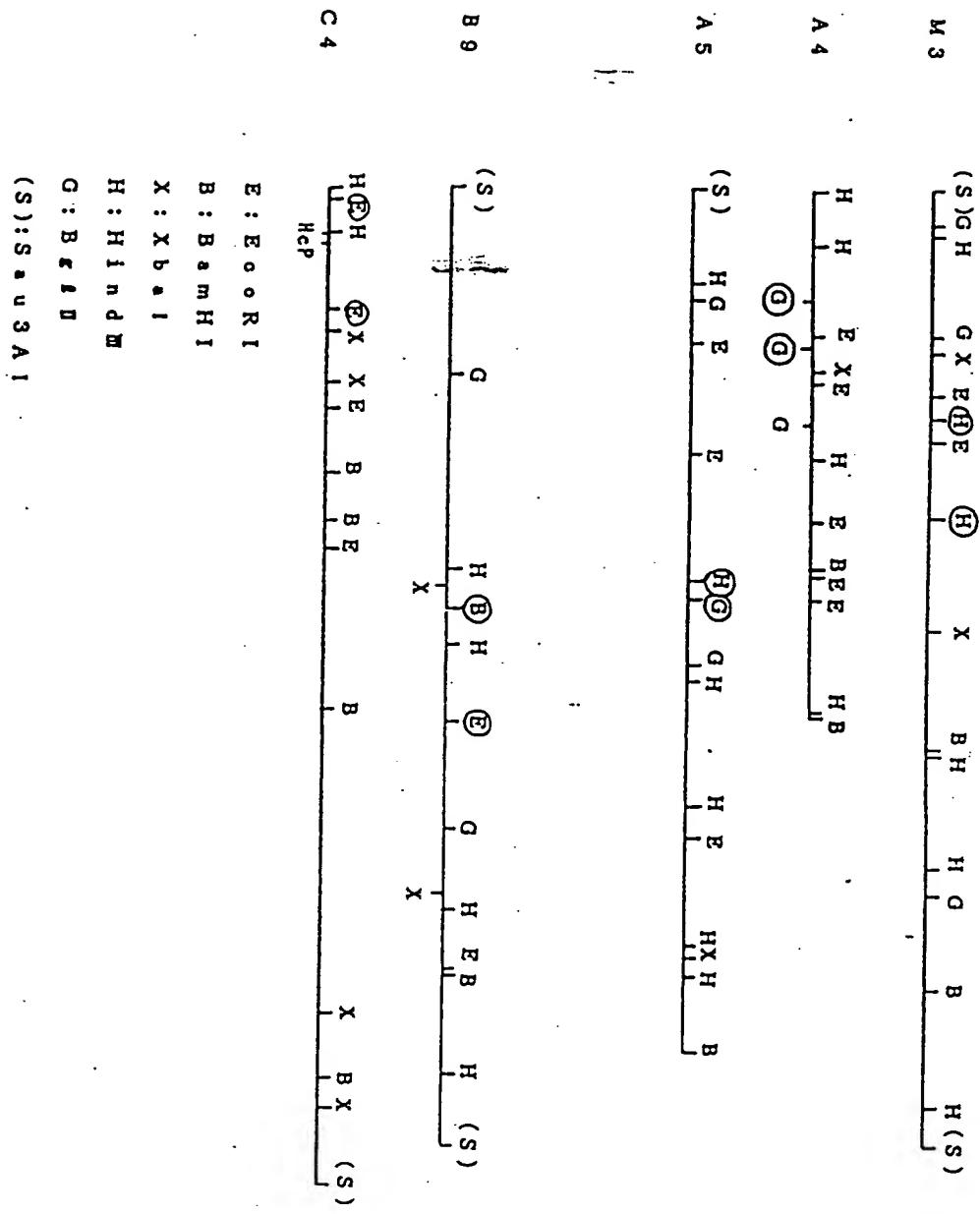
Rabbit chondrocytes were isolated from growth plates of ribs of 3- to 4-week old male New Zealand rabbits, as previously described (Y. Kato et al. *Exp. Cell Res.*). Cells were seeded at a density 10³ cells : 35 mm diameter plastic culture well in 0.1 ml of Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum and antibiotics. When cultures became confluent, the cells were preincubated with 24 hours in 0.1 ml of a 1:1 mixture of DMEM and Ham's F-12 medium supplemented with 0.3% fetal bovine serum (DF). The cells were then transferred to 0.1 ml of the same medium (DF) supplemented with 1 or 5 µl of the medium that was conditioned by various COS cells. (The conditioned medium was diluted or not diluted with DMEM (a final concentration of 10 or 30%). After 3 hours, 5 µl of DMEM supplemented with $^{35}\text{SO}_4^{2-}$ was also added, and incubation was continued for a further 17 hours (Y. Kato et al. *Exp. Cell Res.*).

Claims

- 1. A *Xenopus laevis* bone morphogenetic protein.**

2. A *Xenopus laevis* bone morphogenetic protein in accordance with claim 1, wherein said protein is a mature protein containing an amino acid sequence corresponding to amino acid sequence represented by Nos. 15 to 130 of formula (I) shown in Fig. 3, an amino acid sequence represented by Nos. 14 to 127 of formula (II) shown in Fig. 3, an amino acid sequence represented by Nos. 6 to 119 or Nos. 22 to 119 of formula (III) shown in Fig. 3, an amino acid sequence represented by Nos. 6 to 63 of formula (IV) shown in

Fig. 1



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TTTCCAGTctGATTGCAgTgATACTTTACAATACAATAGGAAAGAACAAATA
 AGTATANN⁷AAGTGATT⁸ACATAAGAAGATCGGTA⁹RACNTGATC¹⁰fgtcaant¹¹g
 TCAATCTA¹³GTAAAGCA¹⁴ACTCTAAGNT¹⁵CAT¹⁶TGACCTA¹⁷TGATNTGCCT¹⁸
 TCTG¹⁹GATTCAGGA²⁰CATC²¹AGTACTGGC²²TGA²³TC²⁴AAAGG²⁵GC²⁶
 GTAACTT²⁷GTATT²⁸TG²⁹GGCT³⁰TC³¹AAAGT³²GTCC³³AGG³⁴CT³⁵GC³⁶
 AT³⁷GGAGT³⁸GCAC³⁹AACT⁴⁰GTAGGA⁴¹TGAT⁴²GGAC⁴³AGAG⁴⁴AAAG⁴⁵GT⁴⁶GGAT⁴⁷AC⁴⁸
 AG⁴⁹GGAT⁵⁰TGAG⁵¹ACAA⁵²ACTG⁵³AACT⁵⁴GTG⁵⁵TT⁵⁶GG⁵⁷AG⁵⁸AAAG⁵⁹GT⁶⁰GGAT⁶¹AC⁶²
 GG⁶³AAAG⁶⁴GT⁶⁵GGAT⁶⁶AC⁶⁷GT⁶⁸GG⁶⁹TC⁷⁰GG⁷¹AAAG⁷²GT⁷³AC⁷⁴
 GCA⁷⁵AAAT⁷⁶CA⁷⁷AC⁷⁸GT⁷⁹AG⁸⁰AT⁸¹GG⁸²AG⁸³GG⁸⁴AG⁸⁵GG⁸⁶CT⁸⁷AC⁸⁸
 CT⁸⁹GT⁹⁰TT⁹¹GG⁹²AAAG⁹³GG⁹⁴AC⁹⁵GT⁹⁶AAAG⁹⁷AC⁹⁸GG⁹⁹GT¹⁰⁰AT¹⁰¹CG¹⁰²
 G¹⁰³AG¹⁰⁴GA¹⁰⁵AG¹⁰⁶AAAG¹⁰⁷GG¹⁰⁸AT¹⁰⁹CA¹¹⁰GG¹¹¹CT¹¹²TC¹¹³GT¹¹⁴AAAG¹¹⁵GG¹¹⁶
 CAGAT¹¹⁷GG¹¹⁸AC¹¹⁹CC¹²⁰AT¹²¹GG¹²²AAAG¹²³GG¹²⁴GT¹²⁵CT¹²⁶AG¹²⁷GT¹²⁸AAAG¹²⁹GT¹³⁰GG¹³¹
 TT¹³²GT¹³³GG¹³⁴AAAG¹³⁵GG¹³⁶AT¹³⁷TC¹³⁸AG¹³⁹GT¹⁴⁰GG¹⁴¹AAAG¹⁴²GT¹⁴³GG¹⁴⁴AT¹⁴⁵CA¹⁴⁶

F i g . 2 (1) - 2

TAGCAGCT⁷⁹⁰GGCTACCA⁸⁰⁰CCAAATT⁸¹⁰TGAGG⁸²⁰GACTGC⁸³⁰
 GAACTACT⁸⁵⁰GGCTCATC⁸⁶⁰TGCTT⁸⁷⁰ACTCC⁸⁸⁰AGT⁸⁹⁰ACAG⁹⁰⁰TAAGG⁹¹⁰
 GCGAAGT⁹¹⁰GGCTTC⁹²⁰ACCG⁹³⁰TGCTGT⁹⁴⁰AGCT⁹⁵⁰AGAG⁹⁶⁰TAAGG⁹⁷⁰
 CCATGTT⁹⁷⁰ACTATG⁹⁸⁰ACGGT⁹⁹⁰AGAA¹⁰⁰⁰AGATA¹⁰¹⁰AGGAT¹⁰²⁰TGA¹⁰³⁰
 TGGAGGA¹⁰³⁰TGCTG¹⁰⁴⁰CATAAGT¹⁰⁵⁰AGAGC¹⁰⁶⁰TGATG¹⁰⁷⁰GCAT¹⁰⁸⁰
 AAATA¹⁰⁹⁰CAATCAAG¹¹⁰⁰AGTGCA¹¹¹⁰GAACAAAG¹¹²⁰TCAAAGT¹¹³⁰TTCAAGG¹¹⁴⁰
 TTRGGC¹¹⁵⁰GAAC¹¹⁶⁰TCACAG¹¹⁷⁰AA¹¹⁸⁰AGCCTG¹¹⁹⁰AG¹²⁰⁰CAATT¹²¹⁰TA¹²²⁰CCAAACG¹²³⁰
 AACCC¹²⁴⁰TTAAGC¹²⁵⁰ACG¹²⁶⁰TGTT¹²⁷⁰AA¹²⁸⁰AAGCAC¹²⁹⁰AA¹³⁰⁰AGAACANA¹³¹⁰
 AAGCTA¹³²⁰TAATG¹³³⁰TCACAT¹³⁴⁰GGACAG¹³⁵⁰AGCT¹³⁶⁰TTACTAC¹³⁷⁰ACATG¹³⁸⁰
 TACANA¹³⁹⁰ATG¹⁴⁰⁰TATAAA¹⁴¹⁰GGTACACT¹⁴²⁰TAAGATA¹⁴³⁰CCAGAGTC¹⁴⁴⁰ATGTTGATGCC¹⁴⁵⁰
 GTRGTCA¹⁴⁶⁰GGCAGT¹⁴⁷⁰AGT¹⁴⁸⁰AGATAA¹⁴⁹⁰ACATG¹⁵⁰⁰GGAAATCTCA¹⁵¹⁰
 TCTTC¹⁵²⁰CTGC¹⁵³⁰ACTATC¹⁵⁴⁰TC¹⁵⁵⁰AAAG¹⁵⁶⁰AAA¹⁵⁷⁰ACAG¹⁵⁸⁰AAA¹⁵⁹⁰AGG¹⁶⁰⁰
 AAATT¹⁵⁷⁰T¹⁵⁸⁰ACGG¹⁵⁹⁰GTGATCA¹⁶⁰⁰AGACTATG¹⁶¹⁰ACTTTG¹⁶²⁰GG¹⁶³⁰TTGAA¹⁶⁴⁰AC¹⁶⁵⁰
 TTACCT¹⁵⁷⁰GAGA¹⁵⁸⁰TTAA¹⁵⁹⁰AGAGACTT¹⁵⁹⁰ACAGT¹⁶⁰⁰GGAA¹⁶¹⁰ACGTA¹⁶²⁰AA¹⁶³⁰TTT¹⁶⁴⁰TG¹⁶⁵⁰
 TCATGAG¹⁶⁶⁰GA¹⁶⁷⁰ACTTG¹⁶⁸⁰AA¹⁶⁹⁰GAACATG¹⁶⁹⁰GG¹⁷⁰⁰GTCCAGG¹⁷¹⁰GG¹⁷²⁰GTGATCC¹⁷³⁰

FIG. 2 (2)

M 3

AGCTTAGAG¹TTGGAA²TG³GATGG⁴A⁵TGCCAATGC⁶
 CAA⁷T⁸CCAC⁹AGGC¹⁰A¹¹GTG¹²A¹³GG¹⁴A¹⁵GTG¹⁶A¹⁷GC¹⁸
 T¹⁹G²⁰T²¹AGG²²A²³T²⁴GG²⁵ATG²⁶A²⁷GG²⁸A²⁹GG³⁰
 G³¹T³²A³³T³⁴GG³⁵C³⁶TTG³⁷CCAA³⁸AG³⁹GG⁴⁰ATC⁴¹GG⁴²A⁴³GG⁴⁴
 CT⁴⁵CATTC⁴⁶GA⁴⁷GG⁴⁸T⁴⁹TA⁵⁰ATC⁵¹GG⁵²CAAT⁵³T⁵⁴CG⁵⁵AC⁵⁶GG⁵⁷
 C⁵⁸T⁵⁹C⁶⁰AG⁶¹GG⁶²CT⁶³GG⁶⁴AT⁶⁵GG⁶⁶CT⁶⁷GG⁶⁸AT⁶⁹GG⁷⁰GT⁷¹GG⁷²
 C⁷³AA⁷⁴CG⁷⁵TT⁷⁶CA⁷⁷GG⁷⁸AT⁷⁹GG⁸⁰CT⁸¹GG⁸²AT⁸³GG⁸⁴CT⁸⁵GG⁸⁶
 C⁸⁷TTGG⁸⁸GC⁸⁹AT⁹⁰CA⁹¹GG⁹²AT⁹³GG⁹⁴CA⁹⁵GG⁹⁶AA⁹⁷GG⁹⁸AT⁹⁹CC¹⁰⁰
 CATTAT¹⁰¹GG¹⁰²TCG¹⁰³GA¹⁰⁴T¹⁰⁵GG¹⁰⁶AC¹⁰⁷GG¹⁰⁸AA¹⁰⁹GG¹¹⁰AT¹¹¹CA¹¹²
 TCACTT¹¹³AA¹¹⁴TT¹¹⁵CA¹¹⁶GG¹¹⁷GA¹¹⁸AT¹¹⁹GG¹²⁰TT¹²¹AC¹²²ANG¹²³AT¹²⁴CA¹²⁵
 GCTG¹²⁶T¹²⁷AG¹²⁸CA¹²⁹AT¹³⁰AN¹³¹CT¹³²TT¹³³GG¹³⁴AT¹³⁵CA¹³⁶GG¹³⁷AT¹³⁸CT¹³⁹

Fig. 2 (3) -1

C 4

EP 0 416 578 A2
 10 20 30 40 50 60
 AGAGCAGGTCAGAGCCCTTGAGTGANAGTGACAGCAGCAAATTGCATCGGATTTAC
 70 80 90 100 110 120
 GACATTGTCAAGGSCAGGGNNNNTGGSCTSCCGGGGCCTTTGAGACTATTGGACA
 130 140 150 160 170 180
 CCAAACACTGGTACATCATATAATGAAAGCAAATGGGAAGTTTGATGTAGCGCCGCCAATTG
 LysLeuValHisHisAsnGluSerLysTrpGluSerPheAspValAlaProAlaileAla
 190 200 210 220 230 240
 CGCGGTGGATTGCACATAAACAGCCTAACCATGGGTTGGTTGTTGAAGTTACTCACTTG
 ArgTrpIleAlaHisLysGinProAsnHisGlyPheValValGluValThrHisLeuAsp
 250 260 270 280 290 300
 ACAATGACAAAATGTGCCTAAGAACGCTATGTGAGGATTAGTAGGTCTAACCCCCGATA
 AsnAspLysAsnValIleProLysHisValArgIleSerArgSerLeuThrProAspLys
 310 320 330 340 350 360
 AAGATAACTGGCCTCAGATACGGCCATTGTTGGTAACCTTTAGCCATGATGGTAAGGAC
 AspAsnTrpProGlnIleArgProLeuLeuValThrPheSerHisAspGlyLysGlyHis
 370 380 390 400 410 420
 ATGCTCTTCACAAAGACAAAGCGCCAAAGCTAGGCACAAACCAACGTTAACGCCCTTAAT
 AlaLeuHisLysSerArgGlnLysSerGlnAlaArgHisLysGinArgLysArgLeuLysSer
 430 440 450 460 470 480
 CGAGCTGCAAGGAGGCATCCGGTTGTTACGTTAGATTCAGCGGACGTTGGGAATGACTGG
 SerCysArgArgHisProLeuTyrrValAspPheSerAspValGlyTrpAsnAspTrpIle
 490 500 510 520 530 540
 TTGTTGCCCAACCTGGGTATCATGCCTTTACTGCCCACGGGGAATGTCCTTCCACTGG
 ValAlaProProGlyTyrrHisAlaPheTyrcyHisGlyGluCysProPheProLeuAla

F i g . 2 (3) - 2

CAG ACC ATT AACT CTACAA ACC ATG CAAT CGT AC AA CCT TT GG TG ACT CT GTC A
 Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser Val Ile Thr
 550 560 570 580 590 600
 CAA AAC AT CCC CAAG GCT TG CT GC GT CC CC AC AGA AACT CAG GCC AT AT CC C AT GCT C T A T C
 Asn Ile Pro Ly s Ala Cy s Cy s Val Pro Thr Glu Leu Ser Ala Ile Ser Met Leu
 610 620 630 640 650 660
 TT GAT GAG AAT GAA AAA AGT AGT ATT AAA AT T AT CA AGA CAT GG TG CG TG AG GG GT GG
 Asp Glu Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp Met Val Val Gln Ile Gly
 670 680 690 700 710 720
 GATGCCGTTAGGCAGTTACGGCCAGAGACAAGAAAGATGACACTTAATATTCC
 Cy s Arg ***
 730 740 750 760 770 780
 TTTTG GAG ACT AT ATT AT GCT TT GAA AA AT GAT GAA AC ANT ATT TT GAA AT ATT ATT
 790 800 810 820 830 840
 AT GT CT ACACGGAGGAGT GG GAAG CA AA AT ATT TA AT CAG AGA AA AT ATT CC TT TT TAG T
 850 860 870 880 890 900
 TGT AC AT TT TATA AG GG GT TG AC CC CAG CAC AT GAA GT AT A AT GG TC AG GAT TG A
 910 920 930 940 950

FIG. 2 (4)

A
4

CCTGAGANTTAAGAAGTGTGGATTAAACAGAACAGGACGGACCGACCAATGAGAAAGCTA
 TTTTCTTGTCTNTGGTAGGACAAAGAACGGGACNTGTTCTTCAAATGAGATTAAAGCCA
 PhePheAsnGluIleLeuAlaArg

130 140 150 160 170 180
 GGTCTGGCCAAAGATGACAAAGACTGTCTATGAATTTCAATCAGAGGAGAAAGAGAC
 SerGlyGlnAspAspLysThrValItyrginutryrLeuPheAsnGinArgArgLysArgArg
 190 200 210 220 230 240
 GAGCTCCTCTGTCAACTAGGCAAGGGAGAGGCCTAATAAGAATTCAAAGCAAGATGTA
 AlaProLeuSerThrArgGlnGlyLysArgProAsnLysAsnSerLysAlaArgCysSer

250 GCAAGAACCACTTCATGTCATTCAAGGATATGGGTTGGATTATTGCC
 Lys Lys Pro Leu His Val Asn Phe Lys Asp Met Gly Trp Asp Asp Trp Ile Ile Ala Pro

310 CTTGGAGTATGAGGCATATCATTGTGAAGGGCTTGAGTTCCCTCTGAGATCT
 320 LeuGluIutYrGiuAlaTyrHisCysGluGlyLeucySGluPheProLeuArgSer
 330
 340
 350

F i g . 2 (5)

A 5

10 AAGCTTACTGGGATCTTCCCATTCCAAGAGGGAAACTTGTTAAGGAGATCA
 20
 30
 40
 50
 60
 70 GGGACAAAGATTAAGTCATAATTGGAAATCCTAAATTCTTGGAGCCACCGGATTCA
 80
 90
 100
 110
 120
 130 GTCCCATCGCCAAGAGGAGATGGAAACGAACACTCTCCCCACTAGGACAATAATGGCAA
 Ser11eAlaLysArgArgTrpLysArgThrThrLeuProThrArgThrAsnGly
 140
 150
 160
 170
 180
 190 AGGTCAATGCCAACAAAGGTAGCAAGGAAGCCCTCTGTCAACTTCAG
 GlyHisAlaLysLysSerLysThrArgCysSerLysLysProLeuLeuValAsnPhelys
 200
 210
 220
 230
 240
 250 GGAGTTGGGTTGGATGACTGGATTATGCTCCCTGGATTATGAAGCCTATCACTGCGA
 GluLeuGlyTrpAspAspTrpIleIleAlaProLeuAspTyrGluAlaTyrHisCysGlu
 260
 270
 280
 290
 300
 310 GGGGGTCTGTGATTTCCTCACTGAGATCT
 GlyValCysAspPheProLeuArgSer
 320

F i g . 2 (6)
p x b r 2 2 (BMP 2A)

10 20 30 40 50 60 70 80 90 100
 GAATTCCTCCCTCACGGCCCTCGTCTACTCACCTCCGGACCCGGCTGAGACTCGCTGCCACTATGTGGACAACTCACCAGA
 110 120 130 140 150 160 170 180 190 200
 CTGGCTGACTGGACGGCGGACTTGTCTCCCTCTGGGACAGCGACTTGAACTAAAGACTCGAGTGATGTGGAAAAAACACGGGGAGAGA
 210 220 230 240 250 260 270 280 290 300
 AAACCCACATCGAGACACAACTCGGCACATAATCGCTCAGGTGACAATGGTGCCTGGATCCACTCTGCTCCGTGCAAGTTAACAGATCTG
 M V A G I H S L L L Q F Y Q I L
 310 320 330 340 350 360 370 380 390 400
 CTGAGCGGCTGCACCCGGCTCGTCCCAGAGGAAGGCAAACGCAAGTATTCCAATCCACTCGCTCGTCCGAGCAGTCCAAACAGTCCTGACCGA
 L S G C T G L V P E E G K R K Y S E S T R S S P Q Q S Q Q V L D Q F
 410 420 430 440 450 460 470 480 490 500
 TTGAGCTCGGCTGCTCAATATGTTGGCTTGAAGAGGGAGGCCAGCCCTGGAAAAATGTTGATCCCCCTACATGTTGGACTTGTACCCCTGCA
 E L R L L N M F G L K R R P T P G K N V V I P P Y H L D L Y H L H
 510 520 530 540 550 560 570 580 590 600
 CTCGGCTCAGTTGGCCATGATCAAGGAAGTTCTGAGGTGGACTATCACATGGAGCGGGCGTAGCAGAGCCAACACAGTGAGGAGCTTCACCATGAA
 S A Q L A D D Q G S S E V D Y H M E R A A S R A N T V R S F H R E
 610 620 630 640 650 660 670 680 690 700
 GAATCCATGGAAGAAAATCCAGAGTCGGTGAGAAAAACATCCAACGATTCTCTCAACCTTCAACATTCAGATGAGGAGCTGGTCACGCTCTG
 E S M E E I P E S G E K T I Q R F F F N L S S I P D E E L V T S S E
 710 720 730 740 750 760 770 780 790 800
 AGCTCGGATTTCGAGAGCAGGTCAAAGGCCATTAAAGACTGACGGCAGCAAACCTCATCGATAAATATTTATGACATTGTCAGGCCAGGGCGG
 L R I F R E Q V Q E P F K T D G S K L H R I N I Y D I V K P A A A
 810 820 830 840 850 860 870 880 890 900
 TGCCTCCGGGGCCCTGTGTAAGACTATTGGACACCAGACTGATCCATCATAATGAAAGCAAATGGAAAGTTGATGTGACGCCGGCAATTACCGG
 A S R G P V V R L L D T R L I H H N E S K V E S F D V T P A I T R
 910 920 930 940 950 960 970 980 990 1000
 TGGATTGCACATAACAGCCTAACCATGGGTTGTTGTAAGTGAAGTCACTTGACAAATGACACAAATGCCCCAGAGGCATGTGAGGATTAGTAGGT
 V I A H K Q P N H G F V V E V T H L D N D T I N V P K R H V R I S R S
 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
 CTTAACCTGGATAAAAGGTCACTGGCTCGGATACGCCATTATGGAACTTITAGCCATGATGGCAAAGGACATGCTCTCACAAAGACAAAACG
 L T L D K G H V P R I R P L L V T F S H D G K G H A L H K R Q K R
 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
 GCAAGCTAGGCACAAACACGTAACGCCCTAAATGGAGCTGAGGGCATCCGTGTAACGTAGATTTCAGTGACGTGGTGGAAATGACTGGATGTT
 Q A R H K Q R K R L K S S C R R H P L Y V D F S D V G W N D V I V
 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
 GCCCCACCTGGGTATCATGCCCTTACTGCCACGGGAAATGTCCTTCCACTGGCAGACCATTAAACTCTACAAACCATGCAATCGTACAAACTTGG
 A P P G Y H A F Y C H G E C P F P L A D H L N S T N H A I V Q T L V
 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
 TGAATTCCGTCAACAAACATCCCAAAGCTTGTGCGTCCCCACAGAACTCACTGGCATCTCCATGCTCTATCTGATGAGAAATGAAAAAGTAGTATT
 N S V N T N I P K A C C V P T E L S A I S M L Y L D E N E K V V L
 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
 AAAGAATTATCAAGACATGGTCGGAGGGTGGCGGTAGCGGGGACACACAAGCCAGAGACAAGAAAGCTGACACTTAAATTTCTTIG
 K N Y Q D M V V E G C G C R *
 1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
 GAGACTATTTATGCTTGAAGGAAACAAATTATTTGAAATATTTATGCTACACGGAGGCTGGGAAGCAAAATTTAATCAGAGAAAT
 1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
 ATTCCCTTTAGTTGACATTTATAAGGGTTGTACCCAGCACATGAACTATGGTCAGATTCTATTTGTATTIACCACTATAACCACTT
 1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
 TTTAAGGAAAAAAATAGCTGTTGTATTTATGTAATCAACAGAGAAAATAGGGTTGTAATAATGTTACTGAAAGTGTGTTCTTCTTCTTCTT
 TAAATTAGTATAACAGCTGGTTATGGCAAGTTTATTTCTATAAAAGCTAATTCAAGCTATTAGTTATAACTTGTGATGTGTTGGJTC
 1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
 ATGGTAAATCTCCATATTGTGCAATTAAACATGCTTATAATGTAACGAGTCAGTCATTGTGCAAGTGTGCTTGTGCAATTGCAAAATTAGAATTIC
 1910 1920 1930 1940 1950 1960 1970 1980 1990

F i g . 2 (7)
p x b r 2 3 (BMP 2 B)

10 20 30 40 50 60 70 80 90 100
 GGAATTCCGGCCCCACTGAGCTTTCCACACATTTTGTGTCACATGGCTGTCAAGAACATGGAATGTTTCTATGCCCTGTTCTGTCAAGA
 110 120 130 140 150 160 170 180 190 200
 CATCATGATTCCTGGTAACCGAATGCTGATGGTCATTATAAGCCAAGTCTGCTGGAGGCACAACTATGCCAGCTGATACTGACACGGCAAG
 M I P G N R M L M V I L S Q V L L G G T N Y A S L I P D T G K
 210 220 230 240 250 260 270 280 290 300
 AAGAAAGTCGGGGCGACATTCAAGGGAGGAGGTGGCAGGGCTCAGAGCAATGAGCTTGTGGGATTTGGAGGTGACGGTGCTGAGATGTTGGAC
 K K V A A D I Q G G G R R S P Q S N E L L R D F E V T L L Q X F G L
 310 320 330 340 350 360 370 380 390 400
 TCCGCAAGCGGGCCGAGCCCAGTAAGGATGGTGGTICCCGTTATGCCGACCTGTACAGGCTTCAGTCAGCGGAGGAGGAGATGAACCTGCACGA
 R K R P Q P S K D V V V P A Y M R D L Y R L Q S A E E E D E L H D
 410 420 430 440 450 460 470 480 490 500
 TATCAGCATGGAGTACCCGAGACACCCACCGCCGCAACACCGTGGAGGAGCTTCATCACGAGGAACATTGGAGAACCTACCAAGGCACAGAAGAA
 I S M E Y P E T P T S R A N T V R S F H H E E H L E N L P G T E
 510 520 530 540 550 560 570 580 590 600
 AATGGAAATTTCGTTTGTGTCACCTCAGCAGCATCCAGAGAACATGGGTGATTTCTTCAGCAGAACACTGAGACTCTATAGAGAACAAATAGACCAG
 N G N F R F V F N L S S I P E N E V I S S A E L R L Y R E Q I D H G
 610 620 630 640 650 660 670 680 690 700
 GTCCAGCGTGGGATGAGGGTTCCACGGATAAAATATATGAAAGTTATGAAACCCATCACAGCAAACGGACACATGATAAAATAGGCTGCTGGACACGAG
 P A V D E G F H R I N I Y E V M K P I T A N G H M I N R L L D T R
 710 720 730 740 750 760 770 780 790 800
 GGTAAATCCACCAATGTGACACAGTGGAAAGTITGATGTAAGCCCTGCAATTATGAGGTGGACCCCTGGATAAACAGATAACCATGGCTTGCCTT
 V I H H N V T Q V E S F D V S P A I M R V T L D K Q I N H G L A I
 810 820 830 840 850 860 870 880 890 900
 GAGGTCACTCACCTCAACAAAACAAAACCTTATCAGGGGAAGCATGTAAGGATAAGTCGATCTTATTACCTCAAAAGGATGCAAGACTGGTCACAGATGA
 E V I H L N Q T K I Y Q G K H V R I S R S L L P Q K D A D V S Q M R
 910 920 930 940 950 960 970 980 990 1000
 GACCACTTTAATTACATTCAAGCCATGATGGCAGGGGGCATGCACTGACTAGGAGGTCAAAAAGAAGTCACAAACAGCAGAGACCCGTAAAAAAATAA
 P L L I T F S H D G R G H A L T R R S K R S P K Q Q R P R K K N K
 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
 ACACGTGGGGAGACATCTCTTATGTGGATTTCAGCGATGIGGGCTGGATGATTGGATTGGACCTCTGGATACCGCCCTTACTGCCATGG
 H C R R H S L Y V D F S D V G V N D V I V A P P G Y Q A F Y C H G
 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
 GATTGTCCATTCCCTGGCTGATCACCTAACTCAACAACTGCTATTGTACAAACTCTGGTAACCTGTAACTCAAGCATCCAAAAGCATGCT
 D C P F P L A D H L N S T N H A I V Q T L V N S V N S S I P K A C C
 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
 GCGTCCCCACAGAACTGAGTGCTATCTCATGCTTATTGGATGAATATGACAACAGTCGCTCTAAAAACTACCAAGGAGATGGTGGTGGAAAGGGTGTGG
 V P T E L S A I S M L Y L D E Y D K V V L K N Y Q E X V V E G C G
 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
 GTGCCGTTGAGTCAGATCCAACAAAAGACTGTTAACGGCTGGACTCTTCCACTGAACATTCAACCTGACCTTATGACTTTATGACTTTATGTAAT
 C R *
 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
 GTTTTTGACAATATGATCATATTTGACAACAAAATATTATAACTACGTATTAAGAAGAAAAAAATAAGTCATTATTTAAACATAA
 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAACGGAAATC

F i s . 2 (8)
pxbr41 (ver1)

10 20 30 40 50 60 70 80 90 100
 GAATTCCGATATGGAATGTAAAAAATCTGGTGAATTATGGGAAGTCGACACAGACCAACTTCAGCATCTTATCTTGACAAAATGAATGCTTGC
 M N A L T
 110 120 130 140 150 160 170 180 190 200
 AGTAAGAGAAGATTCGCTGCTGCTTTCTTTTACATTCACTGAGTCATCGTCAAATAACAATATGGAGAATGATTCCACTCTAGTTT
 V K R R L P V L L F L F H I S L S S I S S N T I L E N D F H S S F
 210 220 230 240 250 260 270 280 290 300
 GTCCAGAGAAAGACTAAAGGCCACGAAACGGCAGAGAGATTCAAAAAGAGATCTTGAECTTTAGTTGCAACACAGACCAAGGCCATTTACCGGAGA
 V Q R R L K G H E R R E I Q K E I L T I L G L Q H R P R P Y L P E K
 310 320 330 340 350 360 370 380 390 400
 AAAAGAAGTCTGACCATTATCATGATGGATTATACAATGCACTAAATATTGAAGAGATGCACTGCTGAAGATGTTCTACAGCAATAAGCCGATCTC
 K K S A P L F K M D L Y N A V N I E E M H A E D V S Y S N K P I S
 410 420 430 440 450 460 470 480 490 500
 CCTAAATGAAGCTTTCACTGGCCACTGACCAAGAGAAATGGCTTCTTGACATGCCACAGCTTATGAGTTTGCTAATTAGTTGACAATGACAAC
 L N E A F S L A T D Q E N G F L A H A D T V M S F A N L V D N D N
 510 520 530 540 550 560 570 580 590 600
 GAATTGCATAAAAACCTCTATGCCAAAAATTCAAGTTGATCTAACTGATATCCCACATGGAGATGAACTGACAGCCGCTGAATTTCGAATTATAAG
 E L H K N S Y R Q X F K F D L T D I P L G D E L T A A E F R I Y K D
 610 620 630 640 650 660 670 680 690 700
 ATTATGTACAAAATAACGAGACATACCAAGGTACCATCTACCAAGGTGCTTAAGAAGCAAGCCGACAAGATCCTTATCTTCCAGGTAGACTCAAGAAC
 Y V Q N N E T Y Q V T I Y Q V L K K Q A D K D P Y L F Q V D S R T
 710 720 730 740 750 760 770 780 790 800
 CATCTGGGGCACAGAAAAGGGATGGCTGACGTTGATATTACTGCAACTGGTAATCACTGGGTGATGAAACCCACATTACAACCTTGGATGGCAGTTAC
 I V G T E K G V L T F D I T A T G N H V V M N P H Y N L G L Q L S
 810 820 830 840 850 860 870 880 890 900
 GTAGAGAGTATGGATATGCAAAATGTTAATCCCAAGGCTTGTGGGCTTGTGGAAAGAATGGTCTCAAGACAAACAGCCATTATGGTGGCATCTTIA
 V E S M D M Q N V N P R L V G L V G K N G P Q D K Q P F M V A F F K
 910 920 930 940 950 960 970 980 990 1000
 AGACCTCAGATATCCATCTCCGAGTGTCTGATCTACTAGCAATAAGCACTGGAAATCAGGAAAGAGCCAAGACCTACAAGGAGCAAGATAATTACCTCC
 T S D I H L R S V R S T S N K H V N Q E R A K T Y K E Q D N L P P
 1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
 AGCAAAATATTACTGATGGCATCATGCCCCCTGGAAAACGTCGTTTTAAAGCAAGCTGCAAGAAACATGAACTGTTGTAAGTTCCGCGATCTGGT
 A N I T D G I M P P G K R R F L K Q A C K K H E L F V S F R D L G
 1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
 TGGCAAGACTGGATAATTGACCTGAAGGATATGCTGCTACTATTGTGATGGAGAATGTGCTTCCACTTAACCTTICATGAATGCCACAAACCATG
 V Q D V I I A P E G Y A A Y Y C D G E C A F P L N S F M N A T N H A
 1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
 CCATITGACAAACGTTGGTACATTCTTAAACCCAGAGACTGTCCTAAGCATGCTGTCACCAACTCAGCTCAATGGTATTCTGTTTACTITGA
 I V Q T L V H F I N P E T V P K P C C A P T Q L N G I S V L Y F D
 1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
 TGACAGTGCCAATGTTATTAAGAAAATACAAAAATATGGTGGTCAAGCCTGTTGCGATTGACAATAGCAGTTATCTGTTTAAACAGTCATT
 D S A N V I L K K Y K N M V V Q A C G C H *
 1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
 TAATGGTATITGCTTATCGTTATTTAAAGTAGAGATACTTGACCATCACACTTAAAGGATGAAAGATTTGTT
 TTGCAATTCGGAATTG

ପ୍ରକାଶକ

B A	ARQSEEDHPHRRRRR-TGLECDGKVNICCKKQFFVFSFKDIGHNDWI IAPSGYHANYCEGECP SHIAG
B 9(I)	... TDE. KK. --. S. H. Y. S. P. D.
M 3(II)	AK-VHEQS. HATK. --S. N. . QNS. L. . R. DYY. D. K. E. . QI. . M. L. . M. . . .
B B	.. LGDSR-. I. K. --. RTSL. . RQ. . ID. RL. T. YG. . . S. . AYL. .
BMP2A	K. EKRQAK. KQ. K. ---. ---. SS-. KRHPLY. D. S. V. . . . V. . P. . . F. . H. . . FPL. D
C 4(III)	K. . KRQAR. KQ. K. --. SS-. RRHPLY. D. S. V. . . . V. . P. . . F. . H. . . FPL. D
DPPC	. . . -HARRPT. . KN---H---DDT-. RRHSY. D. S. V. . DD. . V. . L. . D. Y. . H. K. . FPL. D
Vg 1	TLNPLRCKRP. . K. SYSKLPFTAS. . . K. RHY. E. . V. . QN. V. . Q. . M. . . Y. . . YPLTE
A 4(IV)	R. KRAPLST. --QGKRPNKS. AR-. S. . PLH. N. . M. . D. . . . LE. E. YH. . . L. EFLRS
A 5(V)	R. WKRTTLPT. TNNGK. HAKKS. TR-. S. . PLL. N. . EL. . D. . . LD. E. YH. . . V. DFPLRS
CONSENSE C. F. GW. . W. . . P. . . Y. . . C. G. C.

B A TSGSSLFSFHSTVINHYRMRGHSPFANLKSCCVPTKLRPMMSLYDDGQNIKKDIQNMIVEECGCS
B 9(I) . T. Q. . L. . Q. . TSI. . . . S. . A.
M3(II) A.P. TAA. . . T. . L. LIK--ANNIQTAVN. . . . R. . L. . . F. RNN. VI. T. . AD. . . A. . .
βB VP. . AS. . . TA. V. Q. . . . LN. -GPVN. . . I. . . SS. . . . F. . EY. . V. R. VP. A.
BMP2A H--LNSTN. AI. QTILVN--SVNSK-IP. A. . . . E. SAI. . . L. ENEKVVL. NY. D. V. . G. . R.
C4(III) H--LNSTN. AI. QTILVN--SVNTN-IP. A. . . . E. SAI. . . L. ENEKVVL. NY. D. V. . G. . R.
DPPC H--FNSTN. AV. QTILVN--NMNPGK-VP. A. . . . QLDSVA. . . LN. QSTVVVL. NY. E. T. VG. . R.
Vg1 I--LNG. N. AILQTLVH--SIE. EDIPLP. . . . MS. I. . . F. . NND. VVLRHVE. . A. D. . . R.

CONSENSUS H. C.C. P. M.L. D. M. V. CGC.

F i g . 4 - 1

(VI) BMP2A

M V A G I H S L L L Q F Y Q I L
L S G C T G L V P E E G K R K Y S E S T R S S P Q Q S Q Q V L O Q F
E L R L L N M F G L K R R P T P G K N V V I P P Y X L D L Y H L H
S A Q L A D D Q G S S E V D Y H M E R A A S R A N T V R S F H H E
E S M E E I P E S G E K T I Q R F F F N L S S I P D E E L V T S S E
L R I F R E Q V Q E P F K T D G S K L H R I N I Y D I V K P A A A
A S R G P V V R L L D T R L I H H N E S K V E S F D V T P A I T R
V I A H K Q P N H G F V V E V T H L D N D T N V P K R H V R I S R S
L T L D K G H V P R I R P L L V T F S H D G K G H A L H K R Q X R
Q A R H K Q R K R L K S S C R R H P L Y V D F S D V G V N D V I V
A P P G Y H A F Y C H G E C P F P L A D H L N S T N H A I V Q T L V
N S V N T N I P K A C C V P T E L S A I S K L Y L D E N E K V V L
K N Y Q D K V V E G C G C R *

Fig. 4-2

(V) BMP2B

M I P G N R M L H V I L L S Q V L L G G T N Y A S L I P D T G K
K K V A A D I Q G G G R R S P Q S N E L L R D F E V T L L Q N F G L
R K R P Q P S K D V V V P A Y M R D L Y R L Q S A E E E D E L H D
I S M E Y P E T P T S R A N T V R S F H H E E H L E N L P G T E E
N G N F R F V F N L S S I P E N E V I S S A E L R L Y R E Q I D H G
P A V D E G F H R I N I Y E V M K P I T A N G H M I N R L L D T R
V I H H N V T Q V E S F D V S P A I M R V T L D K Q I N H G L A I
E V I H L N Q T K T Y Q G K H V R I S R S L L P Q K D A D V S Q M R
P L L I T F S H D G R G H A L T R R S K R S P K Q Q R P R K K N K
H C R R H S L Y V D F S D V G W N D V I V A P P G Y Q A F Y C H G
D C P F P L A D H L N S T N H A I V Q T L V N S V N S S I P K A C C
V P T E L S A I S M L Y L D E Y D K V V L K N Y Q E M V V E G C G
C R *

F i g . 4 - 3

(W) (Ver 1)

M N A L T

V K R R L P V L L F L F H I S L S S I S S N T I L E N D F H S S F
V Q R R L K G H E R R E I Q K E I L T I L G L Q H R P R P Y L P E K
K K S A P L F M K D L Y N A V N I E E M H A E D V S Y S N K P I S
L N E A F S L A T D Q E N G F L A H A D T V M S F A N L V D N D N
E L H K N S Y R Q K F X F D L T D I P L G D E L T A A E F R I Y K D
Y V Q N N E T Y Q V T I Y Q V L K K Q A D K D P Y L F Q V D S R T
I V G T E K G V L T F D I T A T G N H V V M N P H Y N L G L Q L S
V E S M D K Q N V N P R L V G L V G K N G P Q D K Q P F M V A F F K
T S D I H L R S V R S T S N K H V N Q E R A K T Y K E Q D N L P P
A N I T D G I M P P G K R R F L K Q A C K K H E L F V S F R D L G
V Q D V I I A P E G Y A A Y Y C D G E C A F P L N S F M N A T N H A
I V Q T L V H F I N P E T V P K P C C A P T Q L N G I S V L Y F D
D S A N V I L K K Y K N M V V Q A C G C H *

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